in cell biology, namely the nature of molecular organization and its spatiotemporal regulation on the plasma membrane.

1588-Pos

Membrane Anchor Dependent Colocalization in Cellular Membranes Observed by Fluorescence Cross-Correlation Spectroscopy

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Membrane anchors exist on many proteins in a variety of combinations of enzymatically attached fatty acids and glypiations. These anchors play a part in protein trafficking within cells and in associating proteins with cell membranes. They are also frequently found on well-known signaling proteins. Given the variety of anchor composition, we question whether these anchors play a more significant role in the lateral sorting or dynamic colocalization of proteins within cell membranes. To observe this *in vivo*, we create fusion proteins of red and green fluorescent proteins with the consensus protein lipidation motif of various signaling proteins and express both red and green constructs in HEK293T cells. The dynamic colocalization of red and green fluorescent proteins, and therefore the dynamic colocalization of membrane anchors, can be directly observed using Fluorescence Cross-Correlation Spectroscopy (FCCS). FCCS allows us to observe dynamic colocalization on the nanometer length scale. Unlike FRET, FCCS can detect positive colocalization regardless of orientation and at lengths larger than 10nm. Recent results will be discussed.

1589-Pos

Growth of the E. Coli Outer Membrane

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The outer membrane (OM) of E. coli is composed of four elements: lipopolysaccharide (LPS), phospholipids, OM proteins, and lipoproteins. Together these elements form a continuous protective layer, defending the bacterium against harsh environments and toxic chemicals. Maintenance of an intact OM requires that synthesis and insertion of new OM components keep pace with bacterial growth. At present, little is known about where new OM is incorporated, or how its growth is regulated. We use video microscopy to examine the behavior of fluorescently labeled LPS and specific OM proteins on the surfaces of growing bacteria. Initially, labeled LPS and OM proteins in an individual cell exhibit a uniform peripheral distribution. As the bacterium elongates, fluorescent spots emerge, subsequently drift apart from one another, and occasionally bifurcate. Arresting bacterial growth with Rifampin halts the motion of the fluorescent spots, resulting in a fluorescence pattern which remains stable over a period of hours. We hypothesize that the appearance and divergence of these fluorescent spots of labeled OM is due to insertion of newly synthesized, unlabeled OM components. We track the motion of these spots on the surfaces of E. coli, and measure the convergence and divergence of adjacent tracks on the periphery of the cell. Our data suggest that new OM is incorporated in patches and distributed non-uniformly, with the bulk of the new material inserted along the lateral walls of the cell and lower rates of insertion in the polar regions of the cell.

1590-Pos

Single-Molecule Study of the Dynamics of Lipid-Like Molecules in the E. Coli Outer Membrane

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While there have been many studies on the diffusion of membrane lipids in eukaryotic cells, which have given insight into the structure and organization of these membranes, little is known to date of their mobility in bacterial membranes, specifically the Gram negative bacteria, *Escherichia coli*. The *E. coli* outer envelope consists of inner and outer lipid membranes that are separated by a periplasmic space containing the cell wall. The outer membrane is unique in that it is thinner than mammalian plasma membranes and consists of a phospholipid inner leaflet with a predominantly lipopolysaccharide (LPS) outer leaflet. Here we look at the diffusion of the fluorescent lipid analog 3,3′-dioctadecylindocyanine iodide (DiI(C_{18})) and Alexa488-LPS in the outer membrane of live E. coli cells using single molecule imaging/tracking techniques. The diffusion coefficient of DiI(C18) was found to be $(5.2 \pm 0.2) \times 10^{-11}$ cm^2/sat time scales of 0.33 *s*. By contrast, the diffusion coefficient of DiI(C18) in human epithelial cancer cells of the nasopharanyx (KB) is found to be $(1.94 \pm 0.2) \times 10^{-8}$ cm^2/s, in good agreement with previously measured diffusion coefficients of DiI(C18) in other mammalian cells. The mobility of LPS in the outer membrane and the implications of the slow diffusion of DiI(C_{18}) on the structure of the outer membrane of *E. coli* will be discussed.

1591-Pos

Investigation of the Confining Potential of Toxin Receptors in Membrane Microdomains by Single Molecule Tracking with Lanthanide-Doped Nanoparticles

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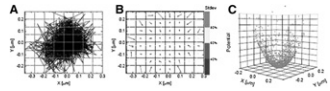

We coupled photostable and non-blinking $Y_{0.6}Eu_{0.4}VO_4$ nanoparticles to epsilon toxins produced by *Clostridium perfringens* type B and D, which bind to a specific receptor on MDCK cells. Single-molecule tracking using these labels shows that the toxin receptor exhibits confined motion within microdomains.

To analyze the receptor trajectories, we introduced a novel approach based on an inference method [1]. Our only assumption is that the receptor moves according to the Langevin equation of motion. This method fully exploits the information of the ensemble of the trajectory (Fig. A), in contrast to the usual mean square displacement analysis, which focuses only on a single observable, the second-order moment. Applying both techniques to collected trajectories, we can highlight the difference in extracted parameters.

From the shape of the confining potential (Fig. C), which is obtained by mapping the forces (Fig. B) inside domains, we can deduce information about the mechanism of confinement. In combination with experiments on cholesterol depletion and cytoskeleton depolymerization, this technique will shed light into the nature of the membrane micropatterning.

[1] J.-B. Masson et al, *Phys. Rev. Lett.***102**, 048103 (2009).

1592-Pos

Mechanisms Regulating the Diffusion of the Lipid Raft Marker Cholera Toxin B Subunit

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The B subunit of cholera toxin (CTXB) is generally accepted as a marker of lipid rafts. Compared to other raft markers or lipid-anchored proteins, CTXB exhibits relatively slow diffusion. A variety of mechanisms could potentially account for this slow diffusion of CTXB, including crosslinking of small raft domains, confinement by the actin cytoskeleton, association with caveolae, incorporation into actively maintained domains, or molecular crowding effects in response to elevated membrane protein density. We evaluated the role of each of these mechanisms in controlling the lateral diffusion of CTXB in the current study by employing fluorescence recovery after photobleaching (FRAP) of fluorescently labeled CTXB following actin depolymerization, ATP depletion, cholesterol depletion, labeling across a range of CTXB concentrations, or in caveolin-1 knockout MEFs. Of these conditions, only cholesterol depletion significantly altered the diffusional mobility of CTXB. Furthermore, we tested whether the slow diffusion of CTXB is an intrinsic property of its receptor by examining the effects of CTXB on the diffusion of a fluorescent GM1 analog. The results of this experiment showed that CTXB slows the diffusion of its receptor. However, binding of CTXB to cells did not affect the diffusion of another raft marker (YFP-GL-GPI), a non-raft marker (YFP-GT46), or a fluorescent lipid analog (DiIC16). Taken together, these data suggest that CTXB diffusion is not limited by actin corrals, caveolae, molecular crowding effects, or the intrinsically slow diffusion of GM1. In addition, they suggest that crosslinking of small rafts induced by CTXB binding does not substantially alter the dynamics of membrane domains enriched in other types of raft or non-raft proteins or lipids.

1593-Pos

Direct Observation of Hop Diffusion of Lipid and Protein Molecules in the Plasma Membrane by High-Speed Single Fluorescent-Molecule Imaging

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